

**SELECTED PHYTOCHEMICAL, NUTRITIONAL
AND ANTIOXIDANT PROPERTIES OF FRESH,
OVEN AND FREEZE-DRIED *Streblus asper*
LEAVES**

by

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Shalawat and remembrance for the Holy Prophet, Muhammad S.A.W.

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at 24 h, 48 h and 72 h incubation

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at 24h, 48h and 72h incubation

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LIST OF ABBREVIATIONS

%	percent
°C	Celsius
μl	microlitre
30% EtOH	30% of ethanol
50% EtOH	50% of ethanol
70% EtOH	70% of ethanol
AAS	Atomic Absorption Spectrometry
AOAC	Association of Official Analytical Chemists
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
Ca	Calcium
cm ²	centimeter square
CO ₂	carbon dioxide
dH ₂ O	distilled water
DPPH	1, 1-diphenyl-2-picrylhydrazyl
FeCl ₃	Iron (III) chloride
g	gram
GCMS	Gas Chromatography Mass Spectrometry
GAE	Gallic acid equivalent
h	hour
H ₂ O ₂	Hydrogen peroxide
H ₂ SO ₄	Sulfuric acid
HBV	Hepatitis B Virus
HCl	Hydrochloric acid
Hg	Mercury
HNO ₃	Nitric acid
HPLC	High Performance Liquid Chromatography
HT 29	Colon carcinoma cell lines
IC ₅₀	Inhibition concentration
K	Potassium
L	liter

LC	Liquid Chromatography
Mg	Magnesium
mg	milligram
min	minute
ml	milliliter
MTS	CellTiter 96® Aqueous One Solution Cell Proliferation Assay
N	Nitrogen
Na	Sodium
Na ₂ CO ₃	Sodium carbonate
NaOH	Sodium hydroxide
nm	nanometer
O ₂	Oxygen
P	Phosphorus
PBS	Phosphate Buffered Saline
QE	Quercetin equivalent
rpm	revolutions per minute
<i>S. asper</i>	<i>Streblus asper</i>
UV	Ultra Violet
v/v	volume per volume
VOCs	Volatile Organic Compounds
w/v	weight per volume

LIST OF PUBLICATIONS

Journal of *Antioxidant* (International Open Access Journal)

Antioxidant Activity and Phenolic Content of *Streblus asper* Leaves Ethanol and Aqueous Extracts from Various Drying Methods

Oral Presentation

1. Antioxidant Activity and Phytochemical Screening of the *Streblus asper* (kesinai) leaf ethanol extracts

Poster Presentation

1. Screening of volatiles in *Streblus asper* leaves by Gas Chromatography-Mass Spectrometry
2. Viability of *Lactobacillus* and Chemical Composition of Cultured Jackfruit Dadih
3. Phytochemicals screening and antioxidant activity of *Streblus asper* (kesinai) leaf in different solvent extracts
4. Antioxidant Activity and Phytochemical Screening of the *Streblus asper* (kesinai) leaf aqueous extracts
5. Cytotoxicity Effect of Cigarette Extract On Fibroblast 3T3 Cell Line and Effect of *Pereskia bleo* on HEP-2 Human Laryngeal Carcinoma Cells
6. Effect of different drying methods on the Proximate Composition, Chlorophyll Contents and Colour of *Streblus asper* (casino) leaves

**MEMILIH FITOKIMIA, NUTRISI DAN CIRI-CIRI ANTIOKSIDA DAUN
SEGAR, PENGERINGAN KETUHAR DAN SEJUKBEKU *Streblus asper***

ABSTRAK

Streblus asper dari famili Moraceae dikenali dengan nama tempatan sebagai kesinai di Malaysia. Herba berubat ini didapati di wilayah utara semenanjung Malaysia seperti Kedah dan Perlis. Kebanyakan kajian berkaitan *S. asper* menggunakan bahagian akar dan kulit pokok. Namun, terdapat dokumentasi tentang kegunaan tradisional daun kesinai sebagai rawatan pembengkakan saluran kencing, penyakit keputihan, keradangan kencing, cirit-birit, melancar pengeluaran susu ibu, dan sebagai agen diuretik. Dalam kerja ini, ciri-ciri fitokimia daun *S. asper* dari pelbagai kaedah pengeringan telah dikaji untuk memperolehi prosedur yang paling sesuai untuk memelihara dan mengekalkan kualiti tinggi tumbuhan perubatan ini tanpa mengurangkan nilai perubatan dan nutrisinya. Daun *S. asper* juga disaring untuk sebatian organik meruap menggunakan GCMS dan penaksiran jumlah asid galik dan kuersetin dilakukan dengan HPLC. Tambahan, kesan pengeringan dan pelarut terhadap sampel terhadap aktiviti antioksidan dalam ekstrak tumbuhan *S. asper* juga ditentukan. Kajian ini menyediakan data asas tentang nilai fizikokimia, nutrisi dan ciri-ciri antioksidan daun *S. asper* daripada pelbagai kaedah pengeringan dan pelarut yang berbeza. Secara umumnya rawatan pengeringan mempengaruhi secara signifikan ($p < 0.05$) kebanyakan komposisi kimia. Keputusan mencadangkan pengeringan sejukbeku adalah lebih efisien dan disyorkan untuk digunakan dalam penyediaan tumbuhan ubatan ini. Dari penyaringan fitokimia, sebatian utama yang telah dikenalpasti dalam *S. asper* adalah fitol, asid lemak, flavonoid, fenolik,

alkaloid, saponin, tanin, terpenoid, glikosida kardiak, klorofil dan juga jumlah ketara mineral, asid galik dan kuersetin. Beberapa unsur lain seperti protin, serat, karbohidrat, asid palmitik, asid linoleik, 12,15-oktadekatrien-1-ol, asid n-heksdekanoik, β -tokoferolerol, vitamin E total telah dikesan dari daun *S. asper*. Kajian ini menunjukkan daun *S. asper* mempunyai aktiviti antioksidan yang baik. Satu pertalian kuat antara fenolik total dan aktiviti penyingkiran radikal telah diperhatikan. Bagaimanapun, ekstrak mentah air dan etanol dari daun *S. asper* tidak menunjukkan kesan kesitotoksikan terhadap sel HT29 secara *in vitro*. Secara amnya, ekstrak pelarut EtOH 70% menunjukkan prestasi lebih baik dari segi aktiviti penyingkiran radikal, kandungan fenolik dan juga kandungan flavonoid berbanding dengan ekstrak air.

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ABSTRACT

Streblus asper from the family Moraceae is known locally as kesinai in Malaysia. This medicinal herb is found in the northern region of peninsular Malaysia especially in Kedah and Perlis. Most studies on *S. asper* concentrate on the root part and stem bark. However, there is documentation on the traditional use of kesinai leaf for the treatment of urinary tract swelling, candidiasis, dysuria, diarrhoea, increase breast milk supply and as diuretic agent. In this research, phytochemical properties of *S. asper* leaves from various drying treatments were studied to get a suitable procedure to preserve and maintain a high quality medicinal plant without reducing its medicinal and nutritive value. The *S. asper* leaves were also screened for VOCs using GCMS and quantification of gallic acid and quercetin were done using HPLC. Additionally, the effects of drying and solvent used for extraction on the level of antioxidant in *S. asper* plant extracts were determined. This study provided preliminary data on the physicochemical, and nutritional values as well as the antioxidant properties of *S. asper* leaves from various drying treatments and solvent extracts. Generally, the drying treatment had significant ($p < 0.05$) effect on most of the chemical constituents. The result suggested that freeze drying was more efficient and is recommended to be used in preparation of this medicinal plant. The compounds identified in *S. asper* is mainly phytol, fatty acids, flavonoids, phenolics, alkaloids, saponin, tannins, terpenoids, cardiac glycosides, chlorophyll; *S. asper* also

contains appreciable amounts of minerals, gallic acid and quercetin. Some of the other phytoconstituents detected in *S. asper* leaves were proteins, fibres, carbohydrates, palmitic acid, linoleic acid, 12,15-octadecatrien-1-ol, n-hexadecanoic acid, β -tocopherol and vitamin E. The study showed that *S. asper* leaves possessed antioxidant activity. A strong correlation between total phenolics and radical scavenging activity was observed. However, crude aqueous and ethanol extracts of *S. asper* leaves did not show any cytotoxicity activity against HT29 cell lines *in vitro*. Generally, 70% EtOH solvent extracts gave better performance and possessed greater radical scavenging activity had higher amounts of phenolic and flavonoid contents compared to aqueous extracts.

CHAPTER 1

INTRODUCTION

1.1 Background

Medicinal plants continue to attract increasing attention because of their potential benefits especially in the field of medicine and pharmacology. Medicinal plants have been recognized for their therapeutic benefits for centuries. Polyphenolic compounds are proven to be potent antioxidants and contain important biological, pharmacological and medicinal properties. The biological activities are antioxidant, anti-proliferative, antibacterial, antifungal, antiviral, anti-diabetic, antihypertensive and anti-inflammatory (Muktar et al., 2005; Arif et al., 2009; Huang et al., 2010). Recently, people have started to look for high-quality dried herbal products that are closely associated with the quality of common raw herbal materials. Several factors contribute to the quality of herbs which are color and drying method. The final color of a dried plant product is a strong factor for marketing.

Drying of herbal plant is done either naturally or by machine. Natural drying is the standard practice that is currently used by most of the Malaysian herbal producers. Machine drying provides higher drying rate and is more hygienic as compared to natural method since it uses heat and operated in a closed chamber. The main purpose of drying is to extend product shelf life (Hamrouni-Sellami et al., 2011) by slowing microorganism growth and preventing certain biochemical reactions that might alter the organoleptic characteristics (Diaz-Maroto et al., 2003; Hamrouni-Sellami et al., 2011).

Recently, most people have started to look into local traditions and natural sources of medicinal plant that may provide potent and safe medicines. They are now starting to be concerned about the side effects of synthetic antioxidants to our health due to enhanced public awareness of the health issues. Natural antioxidants are preferable by consumers and occur naturally from plants, animals, microbial sources, and processed food products. The volume of natural products is increasing day by day and they are available in the market in the forms pills, capsules, liquids and creams; however, the recent trend was “tea infusion” as herbal supplements (Mellgren, 2001). Hence, extensive researches on the use of natural based supplement used are imperative nowadays to identify their biologically active compounds.

Streblus asper Lour (*S. asper*), is an herbal plant known locally as kesinai. *S. asper* (family Moraceae) is found in tropical countries such as Malaysia, Thailand and India. In Thailand and India, this plant has been extensively used in popular folk and Ayurvedic medicine for centuries. However, in Malaysia, there is still limited study and evidence on their typical mechanism of actions and the potential uses of *S. asper* leaf as herbal supplements. This plant is found mainly in surrounding villages and open areas in the northern region of Malaysia. *S. asper* has been used in Malay traditional medicine as decoction and pastes for wound infections. Previously, it has been described and its uses have been identified in Thailand, but only from the bark, stem and root. Thus far, very little is known about on the complete phytochemical and nutritional properties of the leaves. *S. asper* plant has been reported to possess anticancer activity, and to be useful in the treatment of wounds, skin disease, filariasis, leprosy, toothache, diarrhoea, dysentery and especially in the oral cavity